



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>4</sup> :</b>  <b>G01N 33/543, 33/536, 33/569</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 88/ 09933</b>  <b>(43) International Publication Date:</b> 15 December 1988 (15.12.88)
<b>(21) International Application Number:</b> PCT/GB88/00446 <b>(22) International Filing Date:</b> 8 June 1988 (08.06.88) <b>(31) Priority Application Numbers:</b> 8713418 8714213 <b>(32) Priority Dates:</b> 9 June 1987 (09.06.87) 17 June 1987 (17.06.87) <b>(33) Priority Country:</b> GB <b>(71) Applicant (for all designated States except US):</b> WALKER LABORATORIES LTD. [GB/GB]; Cambridgeshire Business Park, Angel Drove, Ely, Cambridgeshire CB7 4DT (GB). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> WALKER, Michael, Strachan [GB/GB]; Walker Laboratories Ltd., Cambridgeshire Business Park, Angel Drove, Ely, Cambridgeshire CB7 4DT (GB). CASBURN-BUDD, Roger, Henry [GB/GB]; 3 Fordham Road, Isleham, Cambridgeshire CB5 8QU (GB).		<b>(74) Agent:</b> GILL JENNINGS & EVERY; 53-64 Chancery Lane, London WC2A 1HN (GB).  <b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> IMMUNOASSAY METHOD  <b>(57) Abstract</b>  A method for detecting antibodies suspected of being present in a specimen, which comprises: a) mixing the specimen and labelled purified antigen with the same purified antigen attached to a solid phase support, to form an immobilised labelled antigen-antibody-attached antigen complex; b) washing away any labelled antigen which is not part of the complex; and c) determining the activity of labelled antigen present on the solid phase.		

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Immunoassay methodField of the Invention

This invention relates to assay systems for the detection of antibodies of all immunoglobulin classes in biological fluids.

Background of the Invention

Mammals, including humans, produce antibodies to invading organisms such as bacteria or viruses. In some diseases, e.g. rheumatoid arthritis, antibodies against the animals own tissues may be produced; these are known as auto-antibodies. The detection of antibodies in serum of patients (human or veterinary) is an important diagnostic procedure in the investigation of infectious diseases, auto-immune diseases, and tolerance to therapy with drug preparations of animal origin, e.g. beef insulin or mouse monoclonal antibodies.

Antibodies are multi-valent, in that they can bind more than one molecule of their specific antigen. This principle has been widely used to detect antibodies (mainly IgM class) by agglutination techniques using red blood cells coated with antigen. The antibodies cause cells to clump or agglutinate together.

Antibodies are also detected by solid phase immunoassays, in which a specific antigen is coated on to a solid phase and then allowed to react with a serum sample suspected of having antibodies present in it. Antibodies which bind to the solid phase may then be detected using a labelled anti-species IgG antibody (e.g. anti-human IgG for patient's serum). The label may be, for example, an isotopic or non-isotopic material, e.g. horseradish peroxidase in the case of an enzymatic label. Enzyme activity detected in the final reaction is proportional to the amount of antibody in the patient's serum. By referring to the activity in pre-calibrated

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standard sera, the antibody content of unknown samples may be quantitated.

In the case of anti-microbial antibodies, IgM antibodies occur in the early stages of the disease and  
5 IgG in later stages. Using current technology, two different assays are normally employed to detect antibodies in early and late infection stages.

In AIDS testing, current methods detect mainly IgG antibodies and can miss IgM antibodies. Therefore,  
10 recent AIDS infections may be missed unless a specific IgM assay is employed.

An object of the present invention is to reduce the complexity and to minimise the disadvantages of existing methods and to produce an immunoassay for the detection  
15 of antibodies which is simple, convenient and able to detect all classes of immuno-globulins simultaneously, to ensure that pathological changes in immune responses are detected in their early stages.

#### Summary of the Invention

20 According to the present invention, a method for the detection of antibodies suspected of being present in a specimen comprises:

- a) mixing the specimen and labelled purified antigen with the same purified antigen attached to a solid phase  
25 support, to form an immobilised labelled antigen-antibody-attached antigen complex;
- b) washing away any labelled antigen which is not part of the complex; and
- c) determining the activity of labelled antigen present  
30 on the solid phase.

The antigen that is labelled and the antigen that is attached to the solid phase are the "same" in the sense that they have the same antigenic site.

The present invention is based on the realisation  
35 that the antigens of multi-valent antibodies will not

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form complexes in the absence of their specific antibodies.

Detection of such antibodies in biological fluids can be achieved in a simple one-step reaction. Clear and  
5 accurate results may be achieved.

All classes of antibodies are detected. An additional advantage is that antibodies from the sera of different animal species may be detected by the same assay, provided that they have a common antigen.

10 Description of the Invention

The present invention involves coating a solid phase support (which may be polystyrene TSP pins or microwells or polystyrene or PVC beads or slides) with purified antigen, any unoccupied binding sites being blocked with  
15 inert proteins.

Diluted sera are then mixed with a solution of purified antigen conjugated to a "label". The label may be an isotope such as <sup>125</sup>I or an enzyme such as horseradish peroxidase or a fluorescent or chemi-  
20 luminescent label.

Once in contact with the antibodies, the labelled and solid phase antigens form immobilised complexes which remain in contact with the solid phase. The period of contact is related to individual assays. Labelled  
25 antigen which is not specifically bound to the solid phase is washed away by a buffer solution.

The amount of labelled antigen bound to the solid phase is determined (e.g. by gamma counting or by incubation with enzyme substrate in the case of non-  
30 isotopic systems). By reference to a standard curve of known amounts of antibody, the unknown samples may be quantified.

The antibodies may be, for example, rheumatoid factors or antibodies to HIV; the process of the  
35 invention may then be used to test for, or monitor,

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rheumatoid arthritis or AIDS in a subject. Rheumatoid factors are anti-globulins and therefore the antigen which is then used in the process of the invention will be a globulin, e.g. IgG.

5       The following Example illustrates the invention.

Example

Human IgG antibody is coated on to the tips of NUNC TSP pins on a plate, at a concentration of 10 µg/ml in a carbonate buffer comprising 0.1 M carbonate, pH 9.6, and  
10 incubated overnight at room temperature. Pins are then blocked with a solution containing 0.5% Bovine Serum Albumin, 5% Lactose and 0.1% Azide, and then dried. Plates can be stored for six months at 4°C.

Next, a plate with 'U' wells is filled with a 1:100  
15 dilution of patients serum, to 50 µl in each well, followed by 50 µl of enzyme-labelled human IgG into all the filled wells. The enzyme is peroxidase. The pins are inserted into the wells and the whole system is incubated for ten minutes at room temperature.

20       The pins are then washed and placed into a flat bottom tray containing 200 µl of a substrate solution comprising o-phenylenediamine in HCl in citrate buffer. They are incubated at room temperature for a further ten minutes. Colour development is stopped by the addition  
25 of 4N sulphuric acid.

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CLAIMS

1. A method for detecting antibodies suspected of being present in a specimen, which comprises:
- a) mixing the specimen and labelled purified antigen
  - 5 with the same purified antigen attached to a solid phase support, to form an immobilised labelled antigen-antibody-attached antigen complex;
  - b) washing away any labelled antigen which is not part of the complex; and
  - 10 c) determining the activity of labelled antigen present on the solid phase.
2. A method according to claim 1, in which the antibodies are rheumatoid factors.
3. A method according to claim 1, in which the
- 15 antibodies are antibodies to HIV.

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# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00446

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>4</sup> : G 01 N 33/543; G 01 N 33/536; G 01 N 33/569																				
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black;">Classification System <sup>1</sup></td> <td style="width: 50%; border-bottom: 1px solid black;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">IPC<sup>4</sup></td> <td style="padding: 5px;">G 01 N 33/00</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup></div>			Classification System <sup>1</sup>	Classification Symbols	IPC <sup>4</sup>	G 01 N 33/00														
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<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category <sup>6</sup></th> <th style="width: 70%; padding: 5px;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; padding: 5px;">Relevant to Claim No. <sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">EP, A, 0161107 (FARMOS-YHTMA OY) 13 November 1985, see page 3, line 8 - page 4, line 13; page 4, line 22 - page 5, line 9; page 8, lines 4-18; page 10, lines 19-22; page 13, line 1 - page 14, line 16 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">GB, A, 2029011 (T.S. BAKER et al.) 12 March 1980, see page 1, lines 77-93; page 1, line 109 - page 2, line 14; page 2, line 98 - page 3, line 48 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">US, A, 4067959 (G. BOLZ) 10 January 1978, see column 1, line 66 - column 2, line 36; column 3, lines 11-18; column 4, lines 35-60; column 6, example 1; column 7, lines 1-17; column 7, lines 51-66 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">US, A, 4189466 (H. AINIS et al.) 19 February 1980, see column 3, line 41 - column 4, line 24; column 13, lines 12-19; figure 4b --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-3</td> </tr> <tr> <td colspan="2" style="text-align: right; padding: 5px;">./.</td> <td></td> </tr> </tbody> </table>			Category <sup>6</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	X	EP, A, 0161107 (FARMOS-YHTMA OY) 13 November 1985, see page 3, line 8 - page 4, line 13; page 4, line 22 - page 5, line 9; page 8, lines 4-18; page 10, lines 19-22; page 13, line 1 - page 14, line 16 --	1	X	GB, A, 2029011 (T.S. BAKER et al.) 12 March 1980, see page 1, lines 77-93; page 1, line 109 - page 2, line 14; page 2, line 98 - page 3, line 48 --	1	X	US, A, 4067959 (G. BOLZ) 10 January 1978, see column 1, line 66 - column 2, line 36; column 3, lines 11-18; column 4, lines 35-60; column 6, example 1; column 7, lines 1-17; column 7, lines 51-66 --	1	Y	US, A, 4189466 (H. AINIS et al.) 19 February 1980, see column 3, line 41 - column 4, line 24; column 13, lines 12-19; figure 4b --	1-3	./.		
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./.																				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>																				
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="padding: 5px;">8th August 1988</td> <td style="padding: 5px;">- 7. 09. 88</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority</td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer</td> </tr> <tr> <td style="padding: 5px;">EUROPEAN PATENT OFFICE</td> <td style="padding: 5px;"> <b>P.C.G. VAN DER PUTTEN</b> </td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	8th August 1988	- 7. 09. 88	International Searching Authority	Signature of Authorized Officer	EUROPEAN PATENT OFFICE	<b>P.C.G. VAN DER PUTTEN</b>										
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International Application No. PCT/GB 88/00446

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Journal of Immunological Methods, vol. 88, no. 1, 1986, Elsevier (Amsterdam, NL), H. Schmitz et al.: "Specific enzyme immunoassay for the detection of antibody to HTLV-III using rheumatoid factor-coated plates", pages 115-120, see page 115, lines 1-9; page 117, column 2, lines 26-38; page 118, figure 1	1-3
X	WO, A, 86/04993 (UNITED STATES DEPARTMENT OF COMMERCE) 28 August 1986, see page 3, lines 2-18; page 8, lines 2-30 -----	1,3

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ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.

GB 8800446

SA 22648

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0161107	13-11-85	GB-A, B 2158578 JP-A- 61034466	13-11-85 18-02-86
GB-A- 2029011	12-03-80	None	
US-A- 4067959	10-01-78	None	
US-A- 4189466	19-02-80	None	
WO-A- 8604993	28-08-86	EP-A- 0196752 AU-A- 5456186 JP-T- 62500049	08-10-86 10-09-86 08-01-87

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